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Occurrence of *Salmonella* in drinking water samples of urban water supply system of Kathmandu

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Abstract

Water-borne diseases are among the leading causes of morbidity and mortality in developing countries and around 2.2 million people die every year due to basic hygiene-related diseases, like gastroenteritis, diarrhea, typhoid and dysentery. Eighty-six water samples were randomly collected from urban water supply system of Kathmandu, and analyzed for physiochemical and microbiological parameters to assess drinking water quality. Residual chlorine was undetectable in 100% samples. *Salmonella* was detected in 4 samples by enrichment culture technique in Selenite F broth followed by plating on Salmonella-Shigella Agar. A total of 10 isolates were identified as *Salmonella* (*S.* Paratyphi, 10% and non-typhi, 90%) by conventional biochemical test. The majority of the isolates were susceptible to most of the antimicrobials tested; however, resistance was observed to amoxicillin (70%), cephalexin (20%) and ceftizoxime (14.28%). There was no significant relationship between coliform and *Salmonella* positivity (P = 0.366). The microbiological quality of urban water supply system is poor and indicates chances of outbreak of *Salmonella* infection.

Key-words: drinking water quality, Nepal, water-borne disease, water pollution.

Introduction

Salmonella belong to the family Enterobacteriaceae. They are motile, Gram negative bacilli that do not ferment lactose, but most of them produce hydrogen sulfide or gas from carbohydrate fermentation. They were originally grouped into more than 2000 species (serotypes) according to their somatic (O) and flagellar (H) antigens (Kauffmann-White classification). Now it is considered that this classification is below species level and that there are no more than 2–3 species (*Salmonella enterica*, *Salmonella bongori* and *Salmonella* Typhi), with the serovars being subspecies. All enteric pathogens except *S.* Typhi are members of the species *S. enterica*. *Salmonella* infection typically causes four clinical manifestations in human: gastroenteritis (ranging from mild to fulminant diarrhea, nausea and vomiting), bacteraemia or septicaemia (high spiking fever with positive blood cultures), typhoid fever/enteric fever (sustained fever with or without diarrhea) and a carrier state in persons with previous infections. In regard to enteric illness, *Salmonella* spp. can be divided into two groups: the typhoidal serovars (*Salmonella* Typhi and *S.* Paratyphi) and the non-typhoidal serovars. *Salmonella* are widely distributed in the environment, but some species or serovars show host specificity. The pathogens typically gain entry into water systems through faecal contamination. Water-borne *Salmonella* outbreaks have devastating public health implications.

Water, although an absolute necessity for life, can be a carrier of many water-borne diseases, such as typhoid, cholera, hepatitis, dysentery and other diarrhea-related diseases. Increasing water demand, shortage of clean drinking water and pollution of water resources are common phenomena of urban development (ENPHO 2003). In Kathmandu Valley, most of the sources of water can't be regarded safe and measured up to the guidelines recommended by WHO (Bottino *et al*. 1991; Prasai 2002). Only 76% of the Nepalese have access to drinking water as against the global average of 87%. Most of the pipe network is old and leaky, while water sources are drying up (The Himalayan Times 2009). The Nepal Water Supply Corporation supplies drinking water to the public which is inadequate and available only for 2 h every alternate day. Water treatment is nonexistent in rural areas and rare in the urban areas. The water disinfected by bleaching powder is ineffective and questionable. Nepal has no drinking water quality standards and loosely follows WHO guidelines (Bhatta *et al*. 2006). This study aims to isolate, identify and characterize salmonellae in drinking water and their risk associated to exposed population.

Materials and Methods

WATER SAMPLING AND ANALYSIS

Eighty-six water samples were randomly collected from different localities of Kathmandu. Samples were collected aseptically in 500 ml bottle containing 1 ml freshly prepared 0.2 N sodium thiosulphate for microbiological analysis. Separate sampling bottles were used for the collection of water sample for detection of residual chlorine. The samples were preserved in an icebox during transportation, brought to the laboratory and analyzed within 6 h of collection (Cheesebrough 2000). Temperature and pH were recorded at the sampling site and the residual chlorine was determined by tritemetic method (Trivedy and Goel 1986).

Table 1. Area-wise distribution of *Salmonella* in drinking water samples of urban water supply system of Kathmandu.

Localities	Code no.	Positive (%)	Total (%)
Kuleshwor	kw1-kw10	0 (0.0)	10 (11.6)
Madannagar	bm1-bm10	0 (0.0)	10 (11.6)
Kirtipur	kp1-kp10	0 (0.0)	10 (11.6)
Teku	t1-t10	0 (0.0)	10 (11.6)
Maitidevi	md1-md10	1 (25.0)	10 (11.6)
Bagbazar	bb1-bb16	2 (50.0)	16 (18.6)
Balkhu	B1-b10	1 (25.0)	10 (11.6)
Gaushala	G1-g10	0 (0.0)	10 (11.6)
Total		4 (100.0)	86 (100.0)

Table 2. Thermotolerant coliform vs. *Salmonella* positivity.

Thermotolerant coliform	Positive sample (%)	Negative sample (%)
0	0 (0.00)	66 (80.50)
2	0 (0.00)	6 (7.30)
4	0 (0.00)	4 (4.90)
8	0 (0.00)	1 (1.20)
12	1 (1.25)	0 (0.00)
16	0 (0.00)	1 (1.20)
18	0 (0.00)	1 (1.20)
25	0 (0.00)	2 (2.40)
26	1 (25.00)	0 (0.00)
28	1 (25.00)	1 (1.20)
52	1 (25.00)	0 (0.00)
Total	4 (100.00)	82 (100.00)

COLIFORM COUNT

Total coliform and thermotolerant coliform were enumerated by the membrane filtration method. Water samples were filtered aseptically through a millipore membrane filter of pore size 0.45 µm. The membrane filter was transferred to M-Endo agar and the plate was incubated at 37ºC (total coliform) and at 44.5ºC (thermotolerant coliform) for 24 h. Total colony forming units were counted after proper incubation (APHA 1995).

ISOLATION AND IDENTIFICATION OF *SALMONELLA*

The water samples (100 ml) were aseptically filtered through a Millipore membrane filter of pore size 0.45 µm. The membrane filters were enriched in 10 ml sterile Selenite F broth for 24 h at 37ºC. A loopful of the suspension from the positive tubes were streaked on the SS agar plates and incubated at 37ºC for 24-48 h. All the pale and the black centered colonies were considered as presumptive *Salmonella* hence purified on Nutrient agar. The pure cultures were identified by the conventional biochemical method (Sneath *et al*. 1986).

ANTIBIOTIC SUSCEPTIBILITY TEST OF *SALMONELLA* ISOLATES

Antibiotic susceptibility test of the isolates were assayed using a modified Kirby-Bauer disk diffusion method. In all the test performed *Escherichia coli* ATCC 25922 was used as reference strain for quality control (NCCLS 1977).

Results

Of 86 water samples analyzed, 4.7% were positive for *Salmonella*. Ten organisms were identified as *Salmonella* by conventional biochemical method; of which 1 (10%) was *S*. Paratyphi A and 9 (90%) were non-typhi. Total coliform was detected in 100% samples and thermotolerant coliform in 23.3% of the sample. Higher percentage (50%) of *Salmonella* was isolated from Bagbazaar area (Table 1). *Salmonella* was found positive in those samples containing total coliform and thermotolerant coliform but all samples containing the coliforms were not always positive for *Salmonella* (Table 2). Antibiotic susceptibility pattern showed that all the isolates were 100% susceptible to tetracycline, chloramphenicol, cotrimoxazole, nalidixic acid and ciprofloxacin, and 70% were resistant to amoxicillin, 20% to cephalexin and 14.28% to ceftizoxime (Table 3). Residual chlorine was undetectable in 100% water samples.

Discussion

Water-borne diseases are among the most emerging and re-emerging disease. It is therefore imperative that safe drinking water is provided. Untreated water is being supplied to most part of the Kathmandu city. The sewer lines and waste water drainage pipelines are laid close to the drinking water distribution lines and chances of cross contamination are higher (Chitrakar and Jackson 2002; Nepal Water Supply Corporation 2004/2005). *Salmonella* was detected in most of the water samples (Chitrakar and Jackson 2002; Bhatta *et al*. 2006). Of 300 samples collected by Bhatta *et al*. (2006), 14% were reported to be positive for *Salmonella* and the organisms were identified as S. Typhi, S. Paratyphi A, S. Typhimurium and S. Enteritidis by conventional biochemical method. In this study, 4 (4.65%) samples were positive for *Salmonella*. Ten organisms were identified as *Salmonella* by the conventional biochemical method, of which 10% were S. Paratyphi A and 90% were non-typhi. *Salmonella* was detected only in those samples that were positive both for total coliform and thermotolerant coliform but not all the samples containing the indicator organisms were positive for *Salmonella*. The presence of *Salmonella* in water samples indicated that the public water supply system of Kathmandu is poor and chances of outbreak of water-borne *Salmonella* infection is higher among people consuming the water without proper disinfection. Occurrence of *Salmonella* in urban water supply system of Nepal is due to old leaky pipes, improper disinfection and cross-contamination (Jackson 2002). The presence of bacteria in water supply system indicates that they might have formed biofilms in the interior of distribution pipelines. Water distribution systems have been reported to provide unique condition for the development of biofilm (Ford 1999; Scher *et al*. 2005). Chances of biofilm formation in Nepalese water distribution system are due to intermittent water supply (Jackson 2002; Nepal Water Supply Corporation 2004/2005).

Chlorine is primarily added to water for destroying the harmful

Table 3. Antibiotic susceptibility pattern of isolates.

S.N	Antibiotics	% Resistant	% Intermidiate	% Sensitive	Total isolates
1.	Amoxycillin	70	30	0	10
2.	Ceftizoxime	10	0	90	10
3.	Cephalexin	20	0	80	10
4.	Cotrimoxazole	0	0	100	10
5.	Nalidixic acid	0	0	100	10
6.	Tetracyclin	0	0	100	10
7.	Ciprofloxacin	0	0	100	10
8.	Chloramphenical	0	0	100	10

microorganisms. Residual chlorine in drinking water after disinfection with chlorinated disinfectant should be 0.2-0.5 mg/l. In this study, residual chlorine was not detected in all samples. Free residual chlorine in the samples from Kathmandu ranged from 0.0 ppm to 1 ppm (Bhatta *et al*. 2006). Kathmandu Upatakya Khanepani Limited has been supplying untreated water in parts of the city, as 23 out of 27 treatment plants were non-functional. Only 13 treatment plants had disinfection facilities. Chlorine content in 47% of piped water samples collected from 120 places in Kathmandu was nil (The Himalayan Times 2009). Traces of free residual chlorine in water indicate that the water is free from germs. There is a lapse of time between treatment and supply so residual chlorine may be lost during storage. The loss in chlorine content in water may be due to the long distance between the place of utilization and the reservoir.

Area-wise distribution of *Salmonella* showed higher percentage (50%) of *Salmonella* in Bagbazaar area. The pipelines of Bagbazaar area are old and leaky at most places and the piped water in the area is turbid. Also the absence of residual chlorine in water samples indicate water contamination with pathogenic microorganisms.

Salmonella was found only in those samples which were positive for coliforms. Similar result was reported by Bhatta *et al*. (2007). Ten *Salmonella* isolates were assayed against 8 different antibiotics. The majority of isolates were susceptible to most of the antimicrobials tested. However, resistance was most commonly directed towards amoxicillin, chephalexin and ceftizoxime respectively. Similarly, Bhatta *et al*. (2007) examined 54 isolates of *Salmonella* spp. for resistance to different antibiotics. Many isolates of *S*. Typhi, *S*. Paratyphi A, *S*. Typhimurium and *S*. Enteritidis were multi drug resistant (MDR) as they were found resistant to at least four antibiotics. All the *S*. Typhi and *S*. Paratyphi A, *S*. Typhimurium and *S*. Enteritidis isolates were found to be sensitive to ciprofloxacin and ofloxacin. All the isolates of *S*. Enteritidis and four isolates of *S*. Typhimurium were resistant to ceftriaxone. No strains of *S*. Typhi and *S*. Paratyphi A were resistant to ceftriaxone. White *et al*. (2003) found that majority of the *Salmonella* isolates were susceptible to antimicrobials tested. However, resistance was observed to tetracycline (26%), streptomycin (23%), sulfamethoxazole (19%), chloramphenicol (8%) and ampicillin (8%). Twenty-eight (36%) *Salmonella* isolates were resistant to at least one antimicrobial and 10 (13%) isolates displayed resistance to four or more antimicrobials. Banani *et al*. (2006) subjected the *Salmonella* isolates to antibiotic sensitivity test. All of the tested isolates were susceptible to ciprofloxacin, ceftriaxone, ceftiofur, ceftizoxime and florfenicol. The percentage of *Salmonella* isolates susceptible to chloramphenicol, sulphamethoxazole-trimethoprime, cephalexin, nalidixic acid, tetracycline, nitrofurantoin, amoxicillin and ampicillin were 92.3, 83.3, 44.9, 25.4, 19.8, 12.4 and 11.1 respectively. From clinical, epidemiological and infection control prospective, it is essential to gather accurate data on all the precise etiological agent of enteric fever because present day vaccines are effective against S. Typhi only and antibiotic susceptibility pattern between serovars potentially differ markedly from one locality to the next (Qu *et al*. 2007). Salmonellosis in human, caused by non-typhoid *Salmonella* strains, usually results in a self-limiting diarrhea that does not warrant antimicrobial therapy. However, occasionally these infection can lead to life-threatening systemic infection that require effective chemotherapy (White *et al*. 2003).

Conclusions

Distinct variation in physicochemical parameters was not observed. Absence of residual chlorine and presence of total coliform in all the samples indicated the likely presence of pathogens. *Salmonella* was present in 4 (4.7%) samples and the isolates were identified as *S*. Paratyphi A and non-typhi. The majority of the isolates were susceptible to most of the antimicrobials tested. However, resistance was observed to amoxicillin (70%), cephalexin (20%) and ceftizoxime (10%). Isolation of *Salmonella* from drinking water and it's resistance to antibiotics indicates the risk associated to the exposed population. This study indicates that quality of piped water of Kathmandu is poor hence strict monitoring in water treatment is needed. Systematic and regular mechanism for surveillance and monitoring of water-borne pathogens in water supply system is a must.

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